EVALUATION OF THE SUSCEPTIBILITY OF A POLYCAPROLACTONE BASED ROOT CANAL FILLING MATERIAL TO MICROBIAL DEGRADATION – AN IN VITRO STUDY

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CERTIFICATE

This is to certify that this dissertation titled "**Evaluation of the susceptibility of a polycaprolactone based root canal filling material to microbial degradation – An in vitro study"** is a bonafide record of work done by **Dr. Lavanya Thangavelu** under my guidance and to my satisfaction during her postgraduate study period between 2009 – 2012. This dissertation is submitted to THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, in partial fulfillment for the award of the degree of Master of Dental Surgery in Conservative Dentistry and Endodontics, Branch IV. It has not been submitted (partial or fully) for the award of any other degree or diploma.

Dr. V. Prabhakar, MDS,
Guide, Professor and Head
Department of Conservative Dentistry and Endodontics,
Sri Ramakrishna Dental College and Hospital, Coimbatore.

Dr. V. Prabhakar, MDS
Principal,
Sri Ramakrishna Dental College and Hospital, Coimbatore.

Date :

Place: Coimbatore.

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CONTENTS

1.	INTRODUCTION	1
2.	AIM AND OBJECTIVE	4
3.	REVIEW OF LITERATURE	5
4.	MATERIALS AND METHODS	23
5.	RESULTS	34
6.	DISCUSSION	53
7.	SUMMARY AND CONCLUSION	58
8.	BIBLIOGRAPHY	59

Introduction

Obturation of the radicular space has been described in various ways for well over 100 years [8]. **Schilder** [39] described the final objective of endodontic procedures as being "the total obturation of the root canal space" in all three dimensions.

In spite of recent advances in visualization, instrumentation and obturation techniques, the use of gutta-percha (a *trans*-1,4-polyisoprene-based thermoplastic material), and a variety of endodontic sealers for filling root canals has remained largely unchanged [17] . In the year 2000, a new thermoplastic resin composite Resilon (Resilon Research LLC, Madison, CT) used with a resin sealer containing difunctional methacrylate resin (Epiphany or RealSeal) was introduced that has the potential to challenge the use of gutta-percha as a root filling material [41,45] . The thermoplasticity of Resilon is attributed to the incorporation of polycaprolactone (PCL), while its bondability with resin cements and self etching primers is attributed to the incorporation of methacrylate based resin components. The other components include bioactive glass, bismuth and barium salts as fillers, and pigments.[32]

Resilon is available as cones for master point and accessory point placement with the lateral condensation technique and as pellets designed for thermoplastic, vertical condensation technique. The main advantage of using this thermoplastic resin as a core material is its ability to bond to methacrylate based resin sealer so that continuous chemical union may be achieved between the root filling material and self etching primer bonded intra radicular dentin. i.e. monoblock concept.

Polycaprolactone which forms the main core for Resilon is a hydrophobic, biocompatible and biodegradable polyester which has been used for bioresorbable sutures, scaffolds and for micro and nanoparticulate drug delivery system [2,12,24,49] . It is reportedly susceptible to both alkaline and enzymatic hydrolysis and is readily degraded and mineralized by a variety of microorganisms [27,30] . The degradation mechanism which has been proposed is hydrolysis of polymer to 6- hydroxyl Caproic acid, an intermediate of omega oxidation and then beta oxidation to Acetyl CoA, which can then undergo further degradation in the tricarboxylic acid cycle. While many methods are available for the in-vitro degradation of polycaprolactone, the most accurate and reproducible method for in-vitro degradation of polycaprolactone is by enzymatic degradation using lipase enzyme [7]. Biodegradation is a process induced by biological activity which results in the change of the chemical structure of the material to naturally occurring metabolic products.

The susceptibility of Resilon to degradation has been studied extensively by **Tay** [14] et al. In their series of studies, it was found that Resilon was susceptible to alkaline hydrolysis after 60 minutes of sodium ethoxide immersion.They also found that Resilon underwent enzymatic biodegradation when tested with two hydrolases - lipase PS and cholesterol esterase. These findings were confirmed by both gravimetric and turbidimetric methods [15,22] . As a sequel to these studies, **Tay**[16] et al further evaluated susceptibility of Resilon to microbial biodegradation by using a simulated field test. The results found surface degradation of the polymer with bacterial and fungal infiltration.

Hiraishi et al [21] found that Resilon was susceptible to biodegradation by cholesterol esterase using agar well diffusion assay of serially diluted aqueous Resilon dispersed in agar. Degradation of the emulsified Resilon was manifested as the formation of clear zones of different sizes around the agar wells.[21]

Investigation of the biodegradability of polymers is a complicated procedure that necessitates the use of a tiered approach [2, 28,18]. Having shown that Resilon is susceptible to abiotic hydrolysis and biotic enzymatic degradation, monoculture screening clear zone tests and simulated field tests under controlled laboratory conditions, the next tier of studies should involve exposure of Resilon to endodontically relevant bacteria and correlate with susceptibility to enzymes produced by these bacteria.

A large variety of aerobic and anaerobic microbes that are present in soil, compost, and sludge [25] can secrete hydrolases such as lipases, esterases, and depolymerases [4] to hydrolyze polycaprolactone and utilize the breakdown products as nutrients [1] . Some of these bacteria such as *Pseudomonas aeruginosa* [23,33], *Enterococcus faecalis* [33,13], and a variety of *Actinomyces*[1] strains have also been isolated from biofilms and sulfur granules that were retrieved from refractory periapical lesions [43].

As these organisms are commonly present in primary, persistant and secondary infection and are capable of producing lipases which cleave the ester bond of polycaprolactone, the present study evaluated the susceptability of Resilon to degradation by endodontically relevant bacteria like *Enterococcus faecalis, Propionibacterium acnes, Actinomyces viscosus, Pseudomonas aeruginosa* and fungi like *Candida albicans*.

Aims and Objective

The aim of this study was to evaluate the susceptability of Resilon to degradation by endodontically relevant bacteria like *Enterococcus faecalis, Propionibacterium acnes, Actinomyces viscosus, Pseudomonas aeruginosa* and fungi like *Candida albicans.*

Review of Literature

Monod J in (1966) [29] gave a Nobel lecture "From enzymatic adaptation to allosteric transitions". This lecture gave the following postulates as *(1)* An allosteric protein is made up of several identical subunits (protomers) *(2)* The protomers are arranged in such a way that none can be distinguished from the others; this implies that there are one or more axes of molecular symmetry and *(3)* Two or more conformational states are accessible to this protein.

Schilder H et al (1967) [39] reviewed the process of endodontic obturation in three dimensions and their techniques and he stated that the final objective of endodontic procedures should be the total three-dimensional filling of the root canals and all accessory canals. In the final analysis, it is the sealing of the complex root canal system from the periodontal ligament and bone which ensures the health of the attachment apparatus against breakdown of endodontic origin. The canal must be shaped to maintain the original anatomical shape so that a continuously tapering funnel is created, with the largest portion coronally. He also stated that the canal system should be filled in three dimensions to within 0.5 to 1 mm of its radiographic apex.

Friedman CM et al (1975) [17] studied the composition and mechanical properties of gutta-percha endodontic points. Gutta-percha endodontic filling points were found to contain approximately 20% gutta-percha (matrix), 66% zinc oxide (filler), 11 % heavy metal sulfates (radiopacifier), and 3% waxes or resins (plasticizer). The mechanical properties were indicative of a partially crystalline viscoelastic polymeric material. Gutta-percha formulations have been used as endodontic filling materials for more than 100 years.

Grossman LI (1976) [19] reviewed endodontics from 1776-1976 through "A bicentennial history against the background of general dentistry". Various sciences have contributed to our understanding of the physiology and pathology of the dental pulp during the past 200 years of progress in endodontics. Although the focal infection theory slowed the acceptance of endodontic treatment in this century, the biomechanical concept of treatment and research have recently opened new avenues for treatment and have initiated improvements in medicaments and filling materials.

Pugh D et al (1977) [37] discussed the cytochemical localization of phospholipase A, lysophospholipase in blastospores and hyphae of *Candida albicans* and the possible role of these fungal enzymes in the invasion of the chick embryonic membrane .

Borssen E and Sundquist G (1981) [5] studied and identified *Actinomyces* in infected dental root canals. Out of twenty-five isolated *Actinomyces* strains, four were *A. israelii*, four were *A. naeslundii*, four were *A. odontolyticus*, and six were *A. viscosus*. Seven strains could be identified only as *Actinomyces* species. These infections could usually be eliminated by one or two conventional endodontic treatments but in two cases alone where *A. israelii* was present, the infection had to be eliminated by means of periapical surgery.

Pitt CG et al (1981) [35] studied aliphatic polyesters and their degradation of polycaprolactone in vivo. The rate of the first stage of the degradation process, non-enzymatic random hydrolytic chain scission, varied by an order of magnitude and was dependent on morphological as well as chemical effects. Weight loss was generally not observed until the molecular weight had decreased to 15,000 or less and the

commencement of weight loss and the rate of weight loss was greater and the period prior to weight loss was shorter when the comonomer content of copolymers of ε-caprolactone was sufficient to reduce the melting point of ε-caproate sequences to body temperature.

Chivian N (1984) [8] overviewed the changes that have taken place in endodontic treatment over the past 25 years. Although the methods and materials have changed markedly, the basic principles of asepsis, enlargement, and seal remained unchanged. He also stated that with future advances in research and technology, the elusive goal of 100 per cent success may become a reality in this century.

Darney PD et al (1989) [10] evaluated clinically the contraceptive implant Capronor, a single-capsule, biodegradable, subdermal contraceptive that releases levonorgestrel over a 12- to 18-month period.

Ando N et al (1990) [3] investigated the presence and types of bacteria invading the deep layers (0.5-2.0 mm from the surface of the root canal wall) of infected dentin of human root canals , sampling with an anaerobic glove box system by splitting the surfaces of eight freshly extracted teeth. More bacteria were recovered after incubation in an anaerobic glove box than after aerobic incubation in air with 30 per cent carbon dioxide. Among the obligate anaerobic isolates, strains belonging to *Lactobacillus* (30 per cent) and *Streptococcus* (13 per cent) were predominant, followed by *Propionibacterium* (9 per cent). No strains of obligate anaerobic Gram-negative rods were isolated and thus the study concluded that the microflora of deep layers of infected root dentin is somewhat similar to that of the deep layers of carious lesions in coronal dentin.

Nair PN et al (1990) [31] evaluated the intra radicular bacteria and fungi in root -filled, asymptomatic human teeth with therapy resistant periapical lesions. Light and electron microscopy were used to analyze nine therapy-resistant and asymptomatic human periapical lesions and the results of the study showed that six of the nine biopsies had the presence of microorganisms in the apical root canal. Four contained one or more species of bacteria and two revealed yeasts. Among the three cases in which no microorganisms could be encountered, one showed histopathological features of a foreign body giant cell granuloma. These findings suggest that in the majority of root-filled human teeth with therapy-resistant periapical lesions, microorganisms may persist and may play a significant role in endodontic treatment failures. Such lesions may also be sustained by foreign body giant cell type of tissue responses at the periapex of root-filled teeth.

Webb EC et al (1992)[49] proposed the recommendations for the nomenclature committee of the International Union of Biochemistry and Molecular Biology on the nomenclature and classification of enzymes. He classified enzymes based solely on the reaction catalyzed especially in cases where proteins of very different sequences catalyze the same reaction.

Jaeger KE et al (1995)[23] evaluated the substrate specificities of bacterial polyhydroxyalkanoate (PHA) depolymerase and lipases. They analyzed the abilities of several bacterial PHA depolymerases, an esterase of *P. fluorescens* GK 13 and five bacterial lipases to hydrolyze various PHA derivatives and triolein. The dimeric ester of hydroxyhexanoate was the main product of enzymatic hydrolysis of polycaprolactone by *Pseudomonas aeruginosa* lipase.

Schirmer A et al (1995)[40] evaluated the substrate specificities of poly (hydroxyalkaonate) - degrading bacteria and active site studies on the extracellular poly (3-hydroxyoctanoic acid) depolymerase of *Pseudomonas fluorescens* GK13. All the poly (hydroxyalkaonate) depolymerases studied so far have been characterized by the lipase consensus sequence Gly -X-Ser-X- Gly in their amino acid sequence, which is a known sequence for serine hydrolases. When it was replaced by the central residue, Ser-172, in the corresponding sequence Gly-Ile-Ser - Ser-Gly of the extracellular poly(3-hydroxyoctanoic acid) depolymerase of Pseudomonas fluorescens GK13 with alanine, the enzyme lost its activity completely. The results of this mutational experiment indicate that the poly(3-hydroxyoctanoic acid) depolymerase belongs to the family of serine hydrolases.

Prananmuda H et al (1995)[36] evaluated the microbial degradation of a synthetic aliphatic polyester, poly (tetramethylene succinate)(PTMS) which has a high melting point. An ecological study showed that the distribution of polytetramethylene succinate degrading microorganisms in soil environment was quiet restricted compared with the distribution of microorganisms that degrade polycaprolactone. *Actinomycete* strain used in the study showed the highest degrading activity of both PTMS and PCL.

Den Dunnen WF et al (1997) [12] evaluated the long term degradation and foreign-body reaction of poly (DL-lactide-epsilon-caprolactone) bars. This specific biomaterial is used for the construction of nerve guides. It was observed that this copolymer degraded completely within 12 months and that no lactide or epsilon caprolactone crystals were formed. Also the foreign body reaction to the poly bars was mild. Page 12

Amass W et al (1998)[2] reviewed biodegradable polymers, its uses, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradation studies. The review summarizes the uses of biodegradable polymers in terms of their relevance within current plastic waste management of packaging materials, biomedical applications and other uses. The degradation processes, both abiotic and biotic reactions together with the development of biodegradation test methods, particularly with respect to composting are also considered.

Gartiser S et al (1998)[18] assessed several test methods for the determination of the anaerobic biodegradability of polymers. Anaerobic degradation of eight commercially available biodegradable polymers were compared. Two anaerobic tests were done using digestion sludge, according to ISO 11734 and ASTM D.5210-91.The extent of degradation for PCL was found to be lower than other polymers. Also carbon dioxide was demonstrated to be essential for the growth of various anaerobic bacteria.

Murphy CA et al (1998) [30] studied polycaprolactone (PCL), a synthetic polyester with applications in biodegradable plastics, which is degraded by a variety of microorganisms, including fungal phytopathogens.

Sundqvist G et al (1998) [44] determined the microbial flora that were present in teeth after failed root canal therapy and established the outcome of conservative re-treatment. Fifty-four root-filled teeth with persisting periapical lesions were selected for re-treatment. After removal of the root filling, canals were sampled by means of advanced microbiological techniques. The teeth were then retreated and followed for upto 5 years.

The isolate most commonly recovered were bacteria of the species *Enterococcus faecalis*. The overall success rate of retreatment was 74 %.

Arpigny J L and Jaeger K E (1999) [4] classified bacterial esterases and lipases based on comparison of their amino acid sequences. The classification enables us to predict (1) important structural features such as residues forming the catalytic site or the presence of disulphide bonds (2) types of secretion mechanism and requirement for lipase-specific foldases and (3) the potential relationship to other enzyme families. The review contributes to a faster identification and to an easier characterization of novel bacterial lipolytic enzymes.

Masatsugu M et al (1999) [27] studied the degradation of Poly(hexano-6-lactone) (PCL) under environmental conditions, including, soil burial, seawater exposure, and activated sludge exposure. The rate of degradation was found to depend on the draw ratio and crystallinity of the PCL fibers with surface erosion of amorphous regions taking place more readily than crystalline regions. In terms of the degradation mechanism of PCL fiber breakdown, biodegradation i.e. the hydrolysis reaction catalyzed by enzymes secreted by microorganisms, is the dominant reaction.

Pandey A et al (1999) [33] reviewed the realm of microbial lipase in biotechnology. In this review, a comprehensive and illustrious survey was made of the applied aspects of microbial lipases in modern biotechnological practices. Lipases are the most versatile biocatalyst and bring about a range of bioconversion reactions such as hydrolysis, interesterification, esterification, alcoholysis, acidolysis and aminolysis. The review also

describes the microbial sources of lipases, the pivotal role of lipases in the processes and products of the food and flavouring industry and importance to biomedical applications.

Elsner HA et al (2000) [13] studied the potential virulence factors of *Enterococcus faecalis* and *Enterococcus faecium in* blood culture isolates. The study revealed that hemagglutinin and lipase may represent additional virulence factors of *Enterococcus faecalis* but not *Enterococcus faecium.*

Mergaert J et al (2000) [28] studied in vitro biodegradation of polyester based plastic materials by selected bacterial cultures. A simple and rapid in vitro test was designed for the assessment of the biodegradation of polyester-based plastics by selected biodegrading bacterial strains. Degradation by the bacteria was studied in liquid medium with the plastics (films, granules, and injection-molded test bars) as sole sources of carbon and the results were mass loss of plastic samples in less than 3 weeks which yielded excellent partially degraded samples for further analysis.

Abou-Zeid et al (2001) [1] evaluated anaerobic biodegradation of natural and synthetic polyesters. It was demonstrated that natural polyester poly(beta-hydroxybutyrate) PHBV and synthetic polyester poly (epsilon-caprolactone) PCL films were degraded by strains of *Clostridium botulinum* and *Clostridium acetobutylicum* under anaerobic conditions at 35°C and 37°C, respectively. By comparing the degradation rate of the two polymer films under anaerobic conditions with the same strains and mesophilic condition, the results showed that degradation rate under mesophilic conditions was about 25-61 fold for PHBV films and 4-73 fold for PCL films higher than under anaerobic conditions. Thus PCL tends to degrade slowly than natural polyester.

Waltimo TM et al (2001)[48] assessed the phenotypes and randomly amplified polymorphic DNA profiles of *Candida albicans* isolates from root canal infections in a Finnish population. A total of thirty-seven *Candida albicans* isolates were subtyped using phenotypic and genotypic methods. The majority of the isolates, 26 strains, were classifiable into three major phenotypes: 16 isolates (43.2%) belonged to phenotype A1R, six (16.2%) to A1S and four (10.8%) to B1S. The remaining 11 phenotypes represented only a single isolate each. For this purpose two different primers, RSD6 and RSD12 were used to develop a combination randomly amplified polymorphic DNA profile for each isolate; 31 genotypes were noted among the 37 isolates, of which only three pairs of isolates presented with congruent phenotypic and genotypic profiles. The heterogeneity of both the phenotypic and randomly amplified polymorphic DNA profiles of *Candida albicans* isolates from root canal infections is akin to previous reports from other oral and non-oral sources in different geographic locales.

Lefevre C et al (2002) [25] evaluated the interaction mechanism between microorganisms and substrate in the biodegradation of polycaprolactone. In conditions where the polymer surface is colonized and a biofilm is formed (under a low stirring rate), polymer weight loss is limited, whereas total degradation is observed when stirring conditions prevent biofilm formation . Results of the SEM observation of the polymer surface as a function of the degradation time suggests that the crystalline and amorphous phase are degraded at about the same rate in the first case, whereas the amorphous phase is preferentially degraded in the latter.

Jones DS et al (2002) [24] studied the poly (epsilon-caprolactone) and poly (epsilon-caprolactone)-polyvinylpyrrolidone-iodine blends as ureteral biomaterials. They also characterised the mechanical and surface properties, degradation and resistance to encrustation in vitro.

Siqueira JF et al (2002)[42] evaluated the prevalence of *Actinomyces* species, *Streptococci*, and *Enterococcus faecalis* in primary root canal infections by using a molecular genetic method. The checkerboard DNA-DNA hybridization assay allowed the detection of *Streptococci* in 22.6% of the samples, *Actinomyces* species in 9.4%, and *E. faecalis* in 7.5%. The most prevalent species were members of the *Streptococcus anginosus* group. In asymptomatic lesions, the most prevalent species were *S. intermedius* (11.5% of the cases), *E. faecalis* (11.5%), and *S. anginosus* (7.7%). *S. constellatus* was the most prevalent species in pus samples (25.9% of the cases). The other most prevalent species in abscessed teeth were *A. gerencseriae* (14.8%), *S. gordonii* (11.1%), *S. intermedius* (11.1%), *A. israelii* (7.4%), *S. anginosus* (7.4%), and *S. sanguis* (7.4%). *S. constellatus* was the only species positively associated with acute periradicular abscess ($p < 0.01$).

Sunde PT et al **(**2002) [43] investigated the periapical microbiota of 36 teeth with refractory apical periodontitis. None of the teeth had responded to conventional endodontic or long-term (> 6 months), calcium-hydroxide treatment. Eight patients had received antibiotics systemically. After anaerobic culture, a total of 148 microbial strains were detected among 67 microbial species. One of the 36 lesions was culture-negative. Approximately half (51.0%) of the bacterial strains were anaerobic. Gram-positive species constituted 79.5% of the flora. Facultative organisms, such as *Staphylococcus, Enterococcus, Enterobacter, Pseudomonas, Stenotrophomonas, Sphingomonas, Bacillus, or Candida*

species were recovered from 27 of the lesions (75%). Sulfur granules were found in 9 lesions (25%). In these granules *Actinomyces israelii, A. viscosus, A. naeslundii, and A. meyeri* were identified. Other bacterial species, both gram-positive and gram-negative, were detected in the granules as well. The study thus demonstrated a wide variety of microorganisms, particularly gram-positive ones, in the periapical lesions of teeth with refractory apical periodontitis.

Hayashi T et al (2002) [20] in their study found that poly(hexano-6-lactone) (PCL) fibers were enzymatically degraded by a hydrolase in vitro. The extent of degradation of PCL fibers was examined by weight loss, loss of mechanical properties such as tensile strength and ultimate elongation decreases, and visual observations by scanning electron microscopy. The rate of degradation was found to depend on draw ratio and crystallinity of the PCL fibers. The strength loss of PCL fibers in the course of degradation took place faster than the weight loss of PCL fibers.

Zou X et al (2002) [50] studied a gel diffusion assay for visualisation and quantification of chitinase activity. Chitinase activity is usually assayed with radiolabeled or fluorogenic substrates. In this study they developed a simple, inexpensive, nonradioactive gel-diffusion assay for chitinase that can be used to screen large numbers of samples. In this assay, chitinase diffuses from a small circular well cut in an agarose or agar gel containing the substrate glycol chitin, a soluble, modified form of chitin. Chitinase catalyzes the cleavage of glycol chitin as it diffuses through the gel, leaving a dark, unstained circular zone around the well, because the fluorescent dye calcofluor binds only to undigested chitin. Results of these activities can be determined from linear regression

of log-standard enzyme concentration versus the zone diameter of internal standards on each petri dish used for a diffusion assay.

Chavez de Paz LE et al (2004) [6] identified gram positive rods prevailing in teeth with apical periodontitis undergoing root canal treatment. Consecutive root canal samples (RCSs) from 139 teeth undergoing root canal treatment were analyzed prospectively for cultivable microbes. Gram-positive rods in the first RCS submitted after chemo-mechanical preparation were categorised to genus level by selective media and gas-liquid chromatography (GLC), and identified to species level by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). In subsequent samples taken during treatment, Gram-positive rods were also identified, although the number of strains was considerably reduced. Positive associations were observed between members of the genus *lactobacilli* and Gram-positive cocci and the study concluded *Olsenella uli* and *Lactobacillus* spp. predominated over other Gram-positive rods. A possible association exists between *Lactobacillus* spp. and Gram-positive cocci in root canals of teeth with apical periodontitis receiving treatment.

Rocas IN et al (2004) [38] evaluated the association of *Enterococcus faecalis* with different forms of periradicular diseases. Culture studies revealed *Enterococcus faecalis* is occasionally isolated from primary endodontic infections but frequently recovered from treatment failures. This molecular study was undertaken to investigate the prevalence of *Enterococcus faecalis* in endodontic infections and to determine whether this species is associated with particular forms of periradicular diseases. When comparing the frequencies of this species in 30 cases of persistent infections with 50 cases of primary infections, statistical analysis demonstrated that *E. faecalis* was strongly associated with

persistent infections. The average odds of detecting *E. faecalis* in cases of persistent infections associated with treatment failure were 9.1. The results of this study indicated that *E. faecalis* is significantly more associated with asymptomatic cases of primary endodontic infections than with symptomatic ones. Furthermore, *E.faecalis* was much more likely to be found in cases of failed endodontic therapy than in primary infections.

Shipper G et al (2004) [41] evaluated the microbial leakage using *Streptococcus mutans* and *Enterococcus faecalis* in roots filled with a thermoplastic synthetic polymer-based root canal filling material (Resilon) and gutta percha using two filling techniques during a 30-day period and the results by Kruskal-Wallis test showed statistical significance when the groups were compared and found Resilon groups superior to gutta-percha groups ($p < 0.05$).

Teixeira FB et al (2004) [45] evaluated the fracture resistance of endodontically treated teeth filled with either gutta-percha or a new resin-based obturation material Resilon. 80 single canal extracted teeth were divided into three groups: lateral and vertical condensation with gutta-percha, lateral and vertical condensation with Resilon , and a control group with no filling material. The Resilon group displayed significantly higher mean fracture loads than gutta-percha groups independent of the filling technique used. It was concluded that filling the canals with Resilon increased the in vitro resistance to fracture of endodontically treated single-canal extracted teeth when compared with standard gutta-percha techniques.

Delahaye F et al (2005) [11] studied and reviewed *Propionibacterium acnes* infective endocarditis with 11 cases. *Propionibacterium acnes*, a gram positive, anaerobic, skin

commensal bacillus, is too often considered a biologic fluid contaminant, of blood cultures in particular. Its implication has been shown in various infections, including brain abscess, ocular infections, osteitis, and acne. It is also the cause of infective endocarditis. Examination of pathologic specimens by polymerase chain reaction increases the sensitivity and speed of its detection.

Franklin RT et al (2005) [14] evaluated the susceptibility of Resilon to degradation by alkaline hydrolysis. 15-mm diameter disks of Resilon and Obtura gutta-percha were prepared by compressive molding and immersed in 20% sodium ethoxide for 20 or 60 min. These disks were examined using field-emission scanning electron microscopy and energy dispersive X-ray analysis. The results showed that the surface resinous component of Resilon was hydrolyzed by sodium ethoxide and more severe erosion occurred with time. Gutta-percha was unaffected after immersion in sodium ethoxide. As Resilon is susceptible to alkaline hydrolysis, it is possible that enzymatic hydrolysis may occur.

Franklin RT et al (2005) [15] gravimetrically evaluated the susceptibility of Resilon to degradation by enzymatic hydrolysis. Resilon, gutta-percha, and polycaprolactone disks, prepared by compression molding, were incubated in phosphate buffered saline, lipase PS or cholesterol esterase at 37°C for 96 hours. Gravimetric analysis showed that all materials exhibited slight weight gains when incubated in phosphate-buffered saline that can be due to water sorption. Gutta-percha showed similar weight gains in the two enzymes. But Resilon and polycaprolactone exhibited extensive surface thinning and weight losses after incubation in lipase PS and cholesterol esterase. **Orstavik D** et al (2005) [32] evaluated the technical, biological and clinical testing methods of the materials used for root canal obturation. Primary infection or infection secondary to root

filling procedures is the principle cause of apical periodontitis and endodontic failure. It is hinted that clinical studies have a high degree of variability because of the multitude of factors affecting outcome. Moreover, refining case selection and limiting the variables in clinical study designs may provide relevant clinical data with better discriminatory power in the future.

Verreck G et al (2005) [46] developed and characterized a biodegradable drug-loaded nerve guide for peripheral nerve regeneration. Sabeluzole, a nerve growth agent, was selected as model compound. Four biodegradable polymers were selected for this study: a copolymer of polylactic acid and polycaprolactone ; a copolymer of polyglycolic acid and polycaprolactone; a copolymer of PCL/polydioxanone (PDO) and PDO. Based on the physicochemical characterization, all samples showed the absence of crystalline sabeluzole, indicating the formation of an amorphous dispersion. The in vitro release measurements show that the release of sabeluzole is complete, reproducible and can be controlled by the proper selection of the polymer.

Walsh G et al (2005) [47] quantified supplemental enzymes in animal feeding stuffs by radial enzyme diffusion. Methods are described which facilitate quantification of supplemental cellulase, protease and alpha-amylase when added to animal feeding stuffs at normal industrial inclusion levels. The methodology involves the extraction of the enzymes from the feeding stuffs by agitation in buffer followed by quantification of extract activity using radial diffusion techniques. A linear relationship between the diameter of the zone of hydrolyzed substrate and the log of the enzyme activity applied is observed over a broad activity range. The methodology used are technically straightforward and will facilitate determination of enzyme stabilities during processes

such as high-temperature pelleting of feeding stuffs, as well as allowing more rigorous quality control related to enzyme-supplemented animal feeding stuffs.

Coulembier O et al (2006) [9] **studied** controlled ring-opening polymerization of biodegradable aliphatic polyester, especially poly (*β*-malic acid) derivatives**.** Poly (*ε*-caprolactone) (PCL) and polylactides (PLAs). Although these polyesters can be produced by polycondensation, high molecular weight structures have, until now, been produced almost exclusively by ring-opening polymerization (ROP) of the corresponding cyclic monomers. The ability of aluminum alkoxides (AlR_x $(OR')_{3-x}$) and tin(II) bis (2-ethylhexanoate) ($Sn(Oct)_2$) to control the ROP of (di)lactones in terms of molecular parameters has opened the way to a wide range of molecular structures and topologies.

Perry AL et al (2006) [34] studied *Propionibacterium acnes*, a common skin organism, most notably recognized for its role in acne vulgaris. It also causes postoperative and device-related infections and has been associated with a number of other conditions such as sarcoidosis and synovitis, acne, pustulosis, hyperostosis and osteitis, although its precise role as a causative agent remains to be determined. *Propionibacterium acnes* produces a number of virulence factors and is well known for its inflammatory and immunomodulatory properties.

Hiraishi N et al (2007) [22] turbidimetrically evaluated the susceptibility of Resilon to degradation by enzymatic hydrolysis. Biotic biodegradation by two hydrolases, lipase PS and cholesterol esterase was done by measuring the optical density reductions in aqueous emulsions containing dissolved, filtered, surfactant-stabilized polymeric components of Resilon. Results of field-emission scanning electron microscopy of air-dried Resilon and

polycaprolactone emulsions revealed the presence of spherical polymer droplets that appeared deformed, pitted, or much reduced in dimensions after enzymatic hydrolysis.

Tay FR et al (2007) [16] examined if Resilon, was susceptible to microbial biodegradation using a simulated field test by incubating the material in wet dental sludge under mesophilic and aerobic conditions. Bacteria and hyphae-like structures were present on the disk surfaces and disappearance of the polymer matrix was accompanied by exposure of mineral and bioactive glass fillers.

Hiraishi N et al (2008) [21] evaluated if Resilon was susceptible to biodegradation by cholesterol esterase using agar-well diffusion assay of serially-diluted aqueous Resilon emulsions that were dispersed in agar. Emulsions of Resilon and polycaprolactone were prepared with different concentrations and dispersed in agar on culture plates using an agar-well diffusion assay for examination of the degradation of polymeric materials and the results of the study showed degradation of the emulsified Resilon manifested as the formation of clear zones of different sizes around the agar wells. No clear zones were observed in agar wells that contain sterile distilled water as the negative control.

Chitra G et al (2010)[7] evaluated the accelerated in-vitro degradation of polycaprolactone using lipase enzyme. Lipase enzyme degrades PCL into Caproic acid. From this study it was clear that the lipase enzyme with PCL consume more alkali when the sample time is increased. It means that the Caproic acid is released from PCL because of action of lipase. Thus when the sample time is increased the liberation of Caproic acid is also increased.

Maria A W and Hutmacher DW et al (2010) [26] reviewed polycaprolactone (PCL). During the resorbable-polymer-boom of the 1970s and 1980s, PCL was used extensively in the biomaterials field and a number of drug-delivery devices. The superior rheological and viscoelastic properties over many of its aliphatic polyester counter parts renders PCL easy to manufacture and manipulate into a large range of implants and devices. This review also discusses the application of PCL as a biomaterial over the last two decades focusing on the advantages which have propagated its return into the spotlight with a particular focus on medical devices, drug delivery and tissue engineering.

Materials and Methods

MATERIALS USED IN THE STUDY

1. Resilon pellets (Pentron Clinical Technologies, Wallingford, CT, USA.)
2. Chloroform (Hi media)
3. Whatman grade 5 filter paper (Hi media)
4. Plysurf A-210 G (Dai-ichi kogyo, Japan.)
5. Ultrasonic disrupter (Branson Ultrasonic Co- operation, Danburry, CT, USA.)
6. Incubator shaker (Lab line model RSI)
7. Brain Heart Infusion agar (Hi media)

ORGANISMS USED IN THE STUDY

1. Enterococcus faecalis (MTCC -439)
2. Propionibacterium acnes (MTCC- 1951)
3. Actinomyces viscosus (MTCC -7345)
4. Pseudomonas aeruginosa (MTCC -2295)
5. Candida albicans (MTCC -183)

These organisms were obtained from MTCC-Microbial type Culture Collection and Gene bank, Chandigarh.

PREPARATION OF STOCK EMULSION OF RESILON

Two grams of Resilon pellets (Pentron Clinical Technologies. Wallingford, CT, USA.) were dissolved in 50 ml of chloroform to form an emulsion. Fillers were removed from this emulsion using Whatman grade 5, micrometer filter paper (Himedia). The filtrate was mixed with 500 ml of sterile distilled water containing Plysurf A-210 G (Dai-ichi kogyo. Japan), a phosphoric ester type anionic surfactant, and subjected to disruption using ultrasonic disrupter (Branson Ultrasonic Co-operation Danburry, CT, USA.). This sonication procedure resulted in the formation of nanodroplets of Resilon. The conical flasks containing Resilon emulsions were agitated inside an incubator shaker (Lab line model RSI) at 37 degree C for 12 hours to ensure that they were stable prior to subsequent serial dilutions.

PREPARATION OF SERIAL DILUTIONS

1. 100 ml of stock solution containing 4g/ litre of Resilon was transferred to 400 ml of distilled water which resulted in $1/5^{th}$ dilution.
2. 100 ml of $1/5^{th}$ dilution was transferred to 400 ml of distilled water which resulted in $1/25^{th}$ dilution.
3. 100 ml from $1/25^{th}$ dilution was transferred to 400 ml of distilled water which resulted in $1/125^{th}$ dilution.
4. 100 ml of $1/125^{th}$ dilution was transferred to 400 ml of distilled water which resulted in $1/625^{th}$ dilution.

Serial dilutions were done to obtain $1/25^{th}$, $1/125^{th}$, and $1/625^{th}$ dilutions of Resilon.

The respective concentration in wt/vol % was:

2×10^{-1} (Stock).

4×10^{-2} ($1/5^{th}$ dilution).

8×10^{-3} ($1/25^{th}$ dilution).

1.6×10^{-3} ($1/125^{th}$ dilution).

3.2×10^{-4} ($1/625^{th}$ dilution).

Stock and serially diluted emulsion were adjusted to pH 7.2 with 0.1 M NaOH.

PREPARATION OF CULTURE MEDIA

Brain Heart Infusion agar (BHI) (Hi media) having the composition of beef heart infusion agar, calf brain infusion agar, disodium hydrogen phosphate, glucose, peptone, and sodium chloride was used in this study, as it supports the growth of a variety of micro organisms. Three flasks containing 250 ml of double strength BHI agar was prepared. Each 250 ml of stock solution, 250 ml of $1/25^{th}$ diluted Resilon emulsion and 250 ml of $1/625^{th}$ diluted Resilon emulsion was mixed with 250 ml of double strength BHI agar. The above media were autoclaved at 121 degree centigrade for 15 minutes and then poured in sterile petridishes to obtain homogenously turbid agar plates containing the emulsified Resilon polymer with the respective dilutions.

REVIVAL OF LYOPHILISED CULTURE

The ampules were wiped with 70 % alcohol and were opened in a biological safety cabinet designed to protect against inhalation of aerosols and to protect the ampules from external contamination. The culture was transferred aseptically and a few drops of the appropriate broth were added to the dried culture using a Pasteur pipette. The contents were mixed well avoiding frothing, and the suspension was transferred to bottles and plates of appropriate media. The organisms were incubated at 37 degree centigrade

and under the appropriate gaseous conditions. After revival of the lyophilized culture, confirmation of the culture was done. The following tests were done for identification of organism used in the study**.**

CONFIRMATION OF THE CULTURE

ENTEROCOCCUS FAECALIS

Gram stained smear showed gram positive cocci in pairs. Minute pink coloured colonies and non haemolytic grey translucent colonies were observed on MacConkey agar and Blood agar respectively. It was positive for Bile esculin test and growth in the presence of 6.5% sodium chloride.

PSEUDOMONAS AERUGINOSA

Gram stained smear showed gram negative rods. Bluish green pigmented colonies and non lactose fermenting colonies were observed on nutrient agar and MacConkey agar plates respectively. It was oxidase positive and metabolized glucose by oxidative fermentation.

ACTINOMYCES VISCOSUS

Gram stained smear showed gram positive rods with branching filaments. Colony appearance on BHI agar shows 2.5 mm round, opaque white slightly convex smooth colonies. It was catalase positive, indole negative, acetoin negative, urease negative, gelatin negative and esculin hydrolase positive.

PROPIONIBACTERIUM ACNES

Gram stained smear showed gram positive diptheroid like rods. Small, circular, convex, white opaque colonies were observed on BHI agar plates. It was catalase positive, urease negative, glucose fermentation positive, and lactose fermentation negative.

CANDIDA ALBICANS

Gram stained smear showed gram positive spherical and budding cells. Creamy white colonies were observed on BHI agar plates. It was confirmed by germ tube test and chlamydospore formation on corn meal agar.

PREPARATION OF INNOCULUM

The turbidity of the bacterial and fungal suspensions was adjusted using Mc Farlands standard number 0.5 (1.5×10^8 cfu/ml). The study included five groups, each group denoting an organism and one control group which were further subdivided into A, B, C.

A –BHI agar containing stock emulsions of resilon .

B- BHI agar containing1/25 th dilution of resilon emulsion

C -BHI agar containing 1/625 th dilution of the resilon emulsion.

GROUPS	Stock (A)	1/25 (B)	1/625 (C)
1.Enterococcus faecalis	1a	1b	1c
2. Propionibacterium acnes	2a	2b	2c
3.Actinomyces viscosus	3a	3b	3c
4. Pseudomonas aeruginosa	4a	4b	4c
5.Candida albicans	5a	5b	5c
6.Negative control	6a	6b	6c

Using one microlitre inoculating loop the bacterial and fungal suspension were streaked as a straight line on to BHI agar plates containing appropriate amount of Resilon emulsion. The culture plates were incubated at 37^{o}C for 48 hours (aerobic and anaerobic conditions) and observed for the zone of clearance at the end of 24 hours and 48 hours. Propionibacterium acnes was incubated under anaerobic conditions and other organisms under aerobic conditions. The total area of zone of clearance was measured using a Computer Aided Design (CAD) software and the values were given in square mm for 24 hours and 48 hours. The values were subjected to statistical analysis using the one way ANOVA followed by Post hoc test and paired t test.

MATERIALS USED

Fig1 - Resilon Pellets

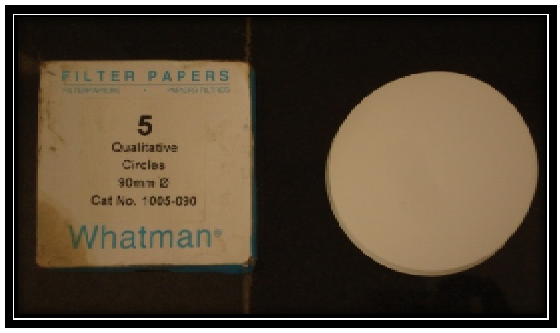


Fig 2 – Whatman Grade 5 Filter Paper

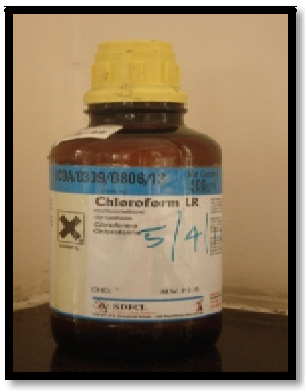


Fig 3- Chloroform

Fig 4 - Plysurf-Surfactant

Fig 5 - Ultrasonic Vibrator

Fig 6- Incubator Shaker

Fig 7 – Brain Heart Infusion

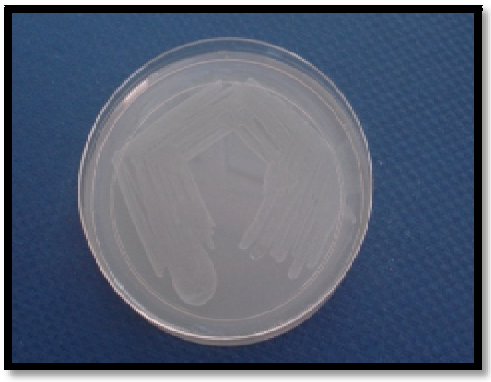


Fig 8 – Culture Plate Of Enterococcus Faecalis

Fig 9- Culture Plate of Propionobacterium Acnes

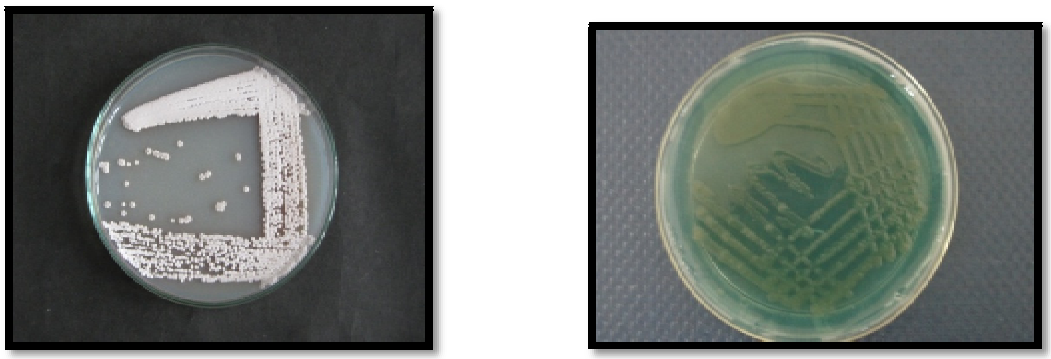


Fig 10 - Culture Plate Of Actinomycosis Viscosus

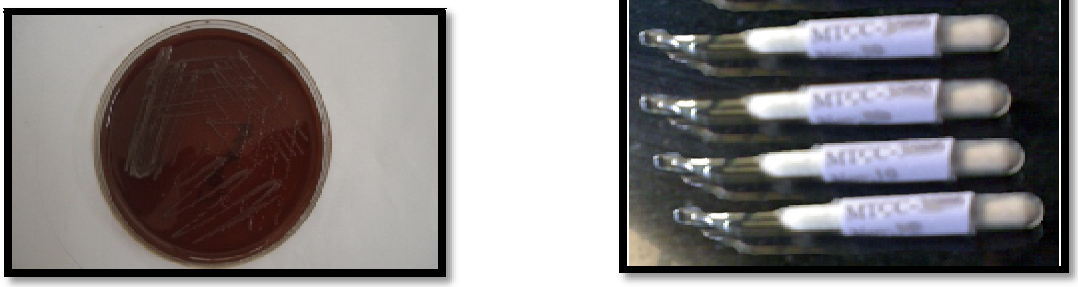


Fig 12- Culture Plate of Candida Albicans

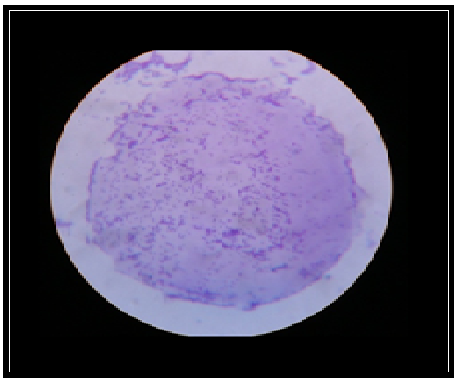


Fig 14- Microscopic Appearance of Enterococcus Faecalis

Fig 15 - Bile Esculin Test- Confirmatory Test For Enterococcus Faecalis

Fig 16- Confirmatory Oxidase Test For Pseudomonas Aeruginosa

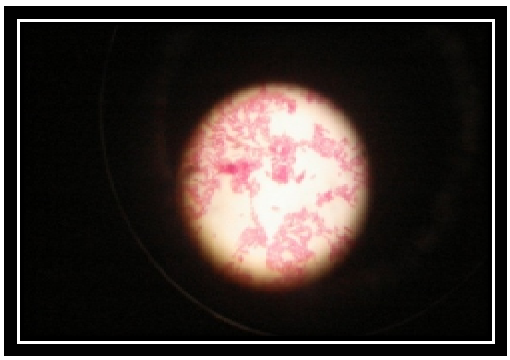


Fig 17- Microscopic Appearance of Pseudomonas Aeruginosa

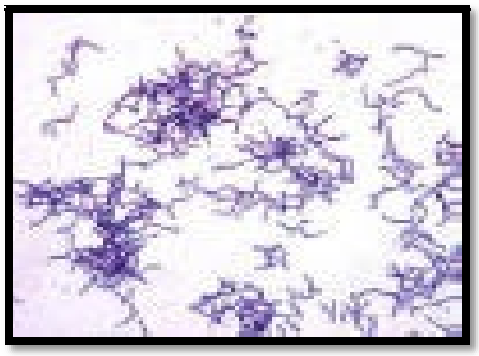


Fig 18- Microscopic Appearance of Actinomycosis Viscosis

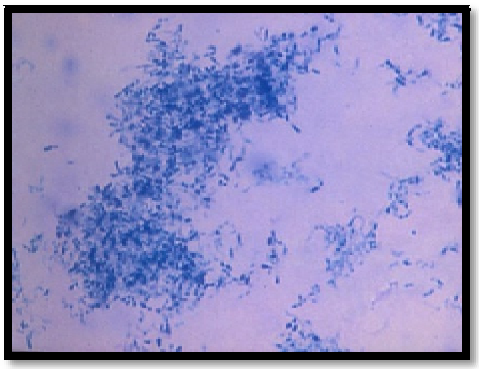


Fig 19- Microscopic Appearance of Propionibacterium Acnes

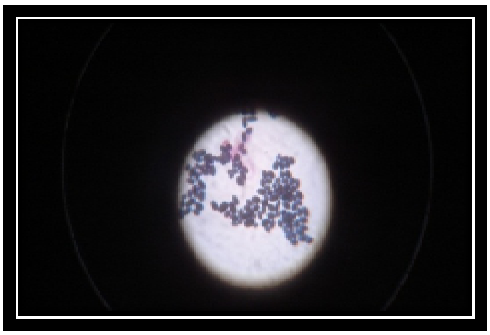


Fig 20- Microscopic Appearance of Candida Albicans

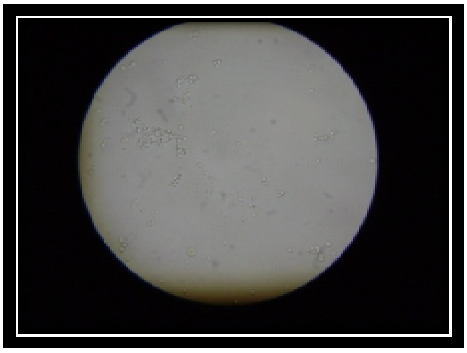


Fig 21- Germ Tube Test For Candida Albicans

Results

Five organisms were tested for zone of clearance on BHI agar incorporated with Resilon suspension to test the hypothesis that Resilon can be degraded by bacteria and fungi. Biodegradable ability of the five organisms were analyzed statistically by one way ANOVA (analysis of variance) followed by Post hoc test and paired t test.

ORGANISM 1 (*Enterococcus faecalis)* in 24 HOURS

TABLE 1a – One way ANOVA showing zone of clearance between the stock and the other 2 dilutions.

	N	Mean	SD	Std.Error
A	3	2.5700	0.01000	0.00577
B	3	3.2467	0.00577	0.00333
C	3	4.9400	0.01000	0.00577
Total	9	3.5856	1.05727	0.35242

TABLE 1b

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	8.942	2	4.471	57485.286	0
With in Groups	0	6	0		
Total	8.943	8			

TABLE 1c - POST HOC TESTS

(I) Sub Type	(J) Sub Type	Mean Difference (I-J)	Std. Error	Sig.
A	B	- .67667*	0.0072	0
	C	-2.37000*	0.0072	0

* The mean difference is significant at the .05 level.

The tables 1a, 1b and 1c indicates that there is a significant difference in the zone of clearance between the stock and the other 2 dilutions. There is a increasingly larger zone of clearance with decreased concentration of the emulsified substrate.

ORGANISM 1 (*Enterococcus faecalis)* in 48 HOURS

TABLE 1d - One way ANOVA showing zone of clearance between the stock and the other 2 dilutions

	N	Mean	SD	Std.Error
A	3	4.17	0.01	0.00577
B	3	6.8267	0.00577	0.00333
C	3	8.25	0.01	0.00577
Total	9	6.4156	1.79341	0.5978

TABLE 1e

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	25.73	2	12.865	165408.1	0
With in Groups	0	6	0		
Total	25.731	8			

TABLE 1f -POST HOC TESTS

(I) Sub Type	(J) Sub Type	Mean Difference (I-J)	Std. Error	Sig.
A	B	- 2.65667*	0.0072	0
	C	- 4.08000*	0.0072	0

* The mean difference is significant at the .05 level.

The tables 1d,1e and 1f indicates that there is a significant difference in the zone of clearance between the stock and the other 2 dilutions. There is a increasingly larger zone of clearance with decreased concentration of the emulsified substrate.

ORGANISM 2 (*Propionibacterium acnes)* in 24 HOURS

TABLE 2a - One way ANOVA showing zone of clearance between the stock and the other 2 dilutions

	N	Mean	SD	Std.Error
A	3	2.03	0.01	0.00577
B	3	2.5	0.01	0.00577
C	3	2.69	0.01	0.00577
Total	9	2.4067	0.29436	0.09812

TABLE 2b

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.693	2	0.346	3463	0
With in Groups	0.001	6	0		
Total	0.693	8			

TABLE 2c- POST HOC TEST

(I) Sub Type	(J) Sub Type	Mean Difference (I-J)	Std. Error	Sig.
A	B	-.47000*	0.00816	0
	C	-.66000*	0.00816	0

* The mean difference is significant at the .05 level.

The tables 2a,2b and 2c indicates that there is a significant difference in the zone of clearance between the stock and the other 2 dilutions. There is a increasingly larger zone of clearance with decreased concentration of the emulsified substrate.

ORGANISM 2(*Propionibacterium acnes)* in 48 HOURS

TABLE 2d- One way ANOVA showing zone of clearance between the stock and the other 2 dilutions

	N	Mean	SD	Std.Error
A	3	3.97	0.01	0.00577
B	3	4.62	0.01	0.00577
C	3	4.96	0.01	0.00577
Total	9	4.5167	0.43572	0.14524

TABLE 2e

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1.518	2	0.759	7591	0
With in Groups	0.001	6	0		
Total	1.519	8			

TABLE 2f- POST HOC TESTS

(I) Sub Type	(J) Sub Type	Mean Difference (I-J)	Std. Error	Sig.
A	B	-.65000*	0.00816	0
	C	-.99000*	0.00816	0

* The mean difference is significant at the .05 level.

The tables 2d, 2e and 2f indicates that there is a significant difference in the zone of clearance between the stock and the other 2 dilutions. There is a increasingly larger zone of clearance with decreased concentration of the emulsified substrate.

ORGANISM 3 (*Actinomyces viscosus)* in 24 HOURS

TABLE 3a- One way ANOVA showing zone of clearance between the stock and the other 2 dilutions

	N	Mean	SD	Std.Error
A	3	4.53	0.01	0.00577
B	3	4.89	0.01	0.00577
C	3	5.86	0.01	0.00577
Total	9	5.0933	0.59582	0.19861

TABLE 3b

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.839	2	1.42	14197	0
With in Groups	0.001	6	0		
Total	2.84	8			

TABLE 3c- POST HOC TESTS

(I) Sub Type	(J) Sub Type	Mean Difference (I-J)	Std. Error	Sig.
A	B	-.36000*	0.00816	0
	C	-1.33000*	0.00816	0

* The mean difference is significant at the .05 level.

The tables 3a,3b and 3c indicates that there is a significant difference in the zone of clearance between the stock and the other 2 dilutions. Increased zone of clearance was produced by organism 3 with decreased concentration of the emulsified substrate in 24 hours.

ORGANISM 3 (*Actinomyces viscosus)* in 48 HOURS

TABLE 3d- One way ANOVA showing zone of clearance between the stock and the other 2 dilutions

	N	Mean	SD	Std.Error
A	3	6.12	0.01	0.00577
B	3	6.63	0.01	0.00577
C	3	6.67	0.01	0.00577
Total	9	6.5733	0.37061	0.12354

TABLE 3e

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1.098	2	0.549	5491	0
With in Groups	0.001	6	0		
Total	1.099	8			

TABLE 3f - POST HOC TESTS

(I) Sub Type	(J) Sub Type	Mean Difference (I-J)	Std. Error	Sig.
A	B	-.51000*	0.00816	0
	C	-.85000*	0.00816	0

* The mean difference is significant at the .05 level.

The tables 3d, 3e and 3f indicate that there is a significant difference in the zone of clearance between the stock and the other 2 dilutions. There is an increasingly larger zone of clearance with decreased concentration of the emulsified substrate.

ORGANISM 4 (*Pseudomonas aeruginosa)* in 24 HOURS

TABLE 4a- One way ANOVA showing zone of clearance between the stock and the other 2 dilutions

	N	Mean	SD	Std.Error
A	3	4.42	0.01	0.00577
B	3	4.87	0.01	0.00577
C	3	5.63	0.01	0.00577
Total	9	4.9733	0.52972	0.17657

TABLE 4b

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.244	2	1.122	11221	0
With in Groups	0.001	6	0		
Total	2.245	8			

TABLE 4c - POST HOC TESTS

(I) Type	(J) Sub Type	Mean Difference (I-J)	Std. Error	Sig.
A	B	-0.45000*	0.00816	0
	C	-1.21000*	0.00816	0

* The mean difference is significant at the .05 level.

The tables 4a,4b and 4c indicates that there is a significant difference in the zone of clearance between the stock and the other 2 dilutions. There is a increasingly larger zone of clearance with decreased concentration of the emulsified substrate.

ORGANISM 4 (*Pseudomonas aeruginosa)* in 48 HOURS

TABLE 4d- One way ANOVA showing zone of clearance between the stock and the other 2 dilutions

	N	Mean	SD	Std.Error
A	3	6.83	0.01	0.00577
B	3	7.15	0.01	0.00577
C	3	8.74	0.01	0.00577
Total	9	7.5733	0.88595	0.29532

TABLE 4e- POST HOC TESTS

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	6.279	2	3.139	31393	0
With in Groups	0.001	6	0		
Total	6.279	8			

TABLE 4f

(I) Type	(J) Sub Type	Mean Difference (I-J)	Std. Error	Sig.
A	B	-.32000*	0.00816	0
	C	-1.91000*	0.00816	0

* The mean difference is significant at the .05 level.

The tables 4d,4e and 4f indicates that there is a significant difference in the zone of clearance between the stock and the other 2 dilutions. There is a increasingly larger zone of clearance with decreased concentration of the emulsified substrate.

ORGANISM 5 (*Candida albicans)* in 24 hours

TABLE 5a- One way ANOVA showing zone of clearance between the stock and the other 2 dilutions

	N	Mean	SD	Std.Error
A	3	5.2	0.01	0.00577
B	3	5.71	0.01	0.00577
C	3	6.4733	0.01	0.00882
Total	9	5.7944	0.55509	0.18503

TABLE 5b

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.464	2	1.232	8529.769	0
With in Groups	0.001	6	0		
Total	2.465	8			

TABLE 5c- POST HOC TESTS

(I) Sub Type	(J) Sub Type	Mean Difference (I-J)	Std. Error	Sig.
A	B	-0.51000*	0.00981	0
	C	-1.27333*	0.00981	0

* The mean difference is significant at the .05 level.

The tables 5a,5b and 5c indicates that there is a significant difference in the zone of clearance between the stock and the other 2 dilutions. There is a increasingly larger zone of clearance with decreased concentration of the emulsified substrate.

ORGANISM 5 (*Candida albicans)* in 48 HOURS

TABLE 5d- One way ANOVA showing zone of clearance between the stock and the other 2 dilutions

	N	Mean	SD	Std.Error
A	3	8.69	0.01	0.00577
B	3	9.71	0.01	0.00577
C	3	10.3567	0.00577	0.00333
Total	9	9.5856	0.72774	0.24258

TABLE 5e

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	4.236	2	2.118	27233.7	0
With in Groups	0	6	0		
Total	4.237	8			

TABLE 5f

Sub Type (I)	(J) Sub Type	Mean Difference (I-J)	Std. Error	Sig.
A	B	-1.02000*	0.0072	0
	C	-1.66667*	0.0072	0

* The mean difference is significant at the .05 level.

The tables 5d,5e and 5f indicates that there is a significant difference in the zone of clearance between the stock and the other 2 dilutions. There is a increasingly larger zone of clearance with decreased concentration of the emulsified substrate.

ORGANISM 1(*Enterococcus faecalis)*

TABLE 6 - T-test showing a significant difference in the zone of clearance produced by *Enterococcus faecalis* in 24 and 48 hours.

	Mean	**N**	**SD**	**Correlation value**	**t value**	**Level of significance**
Area for 24 hours(sq.cm)	3.5867	9	1.05727	0.915	9.13	0.001
Area for 48 hours(sq.cm)	6.4167	9	1.79341			

ORGANISM 2 (*Propionibacterium acnes)*

TABLE 7 - T-test showing a significant difference in the zone of clearance produced by *Propionibacterium acnes* in 24 and 48 hours.

	Mean	**N**	**SD**	**Correlation value**	**t value**	**Level of significance**
Area for 24 hours(sq.cm)	2.4067	9	0.29436	0.998	44.24	0.001
Area for 48 hours(sq.cm)	4.5167	9	0.43572			

ORGANISM 3 (*Actinomyces viscosus)*

TABLE 8 - T-test showing a significant difference in the zone of clearance produced by *Actinomyces viscos us)* in 24 and 48 hours.

	Mean	**N**	**SD**	**Correlation value**	**t value**	**Level of significance**
Area for 24 hours(sq.cm)	5.0933	9	0.59582	0.931	15.58	0.001
Area for 48 hours(sq.cm)	6.5733	9	0.37061			

ORGANISM 4 (*Pseudomonas aeruginosa)*

TABLE 9 - T-test showing a significant difference in the zone of clearance produced by *Pseudomonas aeruginosa* in 24 and 48 hours.

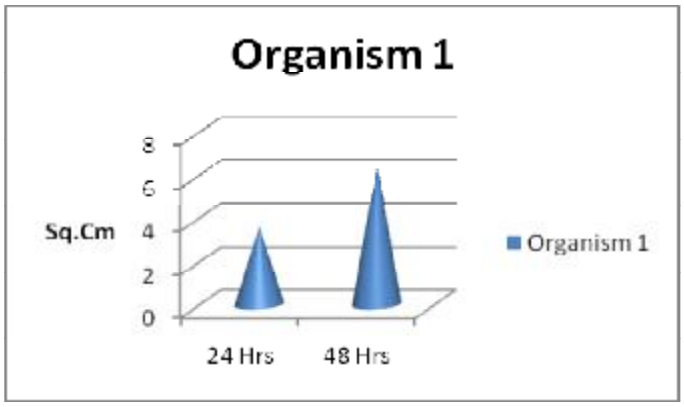
	Mean	**N**	**SD**	**Correlation value**	**t value**	**Level of significance**
Area for 24 hours(sq.cm)	4.9733	9	0.52972	0.976	20.18	0.001
Area for 48 hours(sq.cm)	7.5733	9	0.88595			

ORGANISM 5 (*Candida albicans)*

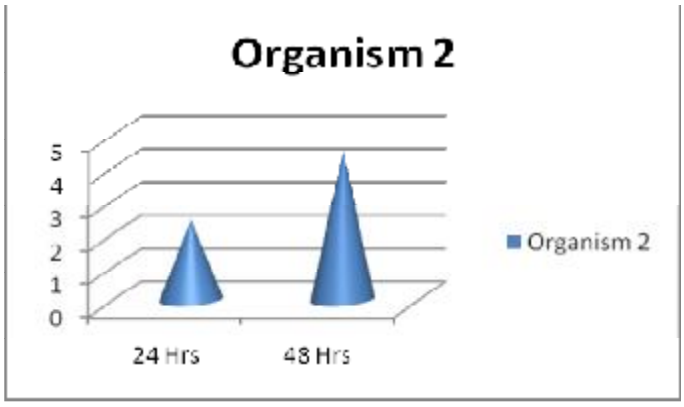
TABLE 10 - T-test showing a significant difference in the zone of clearance produced by *Candida albicans* in 24 and 48 hours.

	Mean	**N**	**SD**	**Correlation value**	**t value**	**Level of significance**
Area for 24 hours(sq.cm)	5.7933	9	0.55509	0.971	49.13	0.001
Area for 48 hours(sq.cm)	9.5867	9	0.72774			

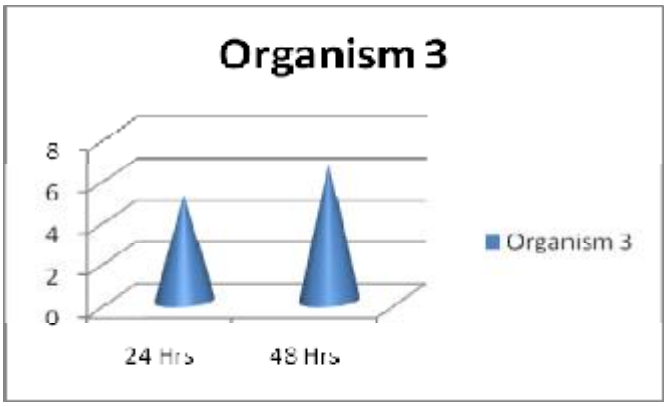
A COMPARISON OF ZONE OF CLEARANCE IN 24 HOURS AND 48 HOURS.



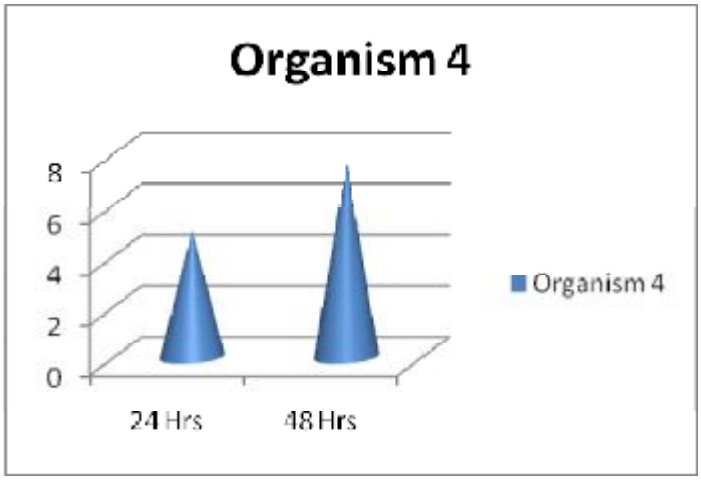
ORGANISM 1-*(Enterococcus faecalis)*



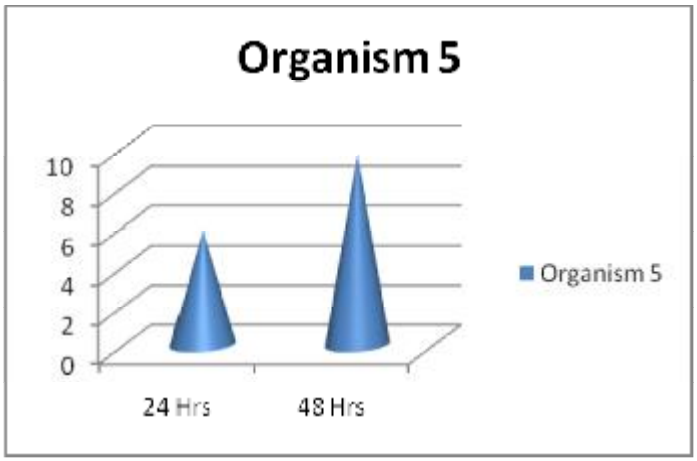
ORGANISM 2-*(Propionibacterium acnes)*



ORGANISM 3-*(Actinomyces viscosus)*



ORGANISM 4-*(Pseudomonas aeruginosa)*



ORGANISM 5 –(*Candida albicans)*

TABLE 11- One way ANOVA comparing zone of clearance produced by the five organisms in 24 hours.

	N	Mean	SD	Std. Error
Organism 1	9	3.587	1.05	0.35242
Organism 2	9	2.407	0.29	0.09812
Organism 3	9	5.093	0.59	0.19861
Organism 4	9	4.973	0.55	0.17657
Organism 5	9	5.797	1.37	0.18503
0.36454	45	4.371	1.37	0.20556

TABLE 12

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	66.476	4	16.619	38.681	0.001
With in Groups	17.186	40	0.43		
Total	83.662	44			

TABLE13 – POST HOC TEST (24 HOURS)

Organism(I)	Organism(J)	Mean Difference (I-J)	Std. Error	Sig.
Organism 1	Organism 2	1.17889	30899	0.013
	Organism 3	-1.50778	0.30899	0.001
	Organism 4	-1.38778	0.30899	0.002
	Organism 5	-2.20889	0.30899	0

* The mean difference is significant at the .05 level.

Tables 11,12 and 13 shows the difference in the zone of clearance produced by the five organisms in 24 hours.In the table 12, the mean value 38.681 for the mean difference in the zone of clearance among the five organisms is significant .($p < .001$).Hence there is a significant difference in the zone of clearance among the five organisms in 24 hours. Further the post hoc test (table 13) reveals that the organism 2 has the less zone of clearance compared to other organisms .The zone of clearance of organism 1,3,4 and 5 are almost similar.

TABLE 14 - One way ANOVA comparing zone of clearance produced by the five organisms in 48 hours.

	N	Mean	SD	Std.Error
Organism 1		6.4156	1.79341	0.5978
Organism 2	9	4.5167	0.43572	0.14524
Organism 3	9	6.5733	0.37061	0.12354
Organism 4	9	7.5733	0.88595	0.29532
Organism 5	9	9.5856	0.72774	0.24258
Total	45	6.9329	1.91881	0.28604

TABLE 15

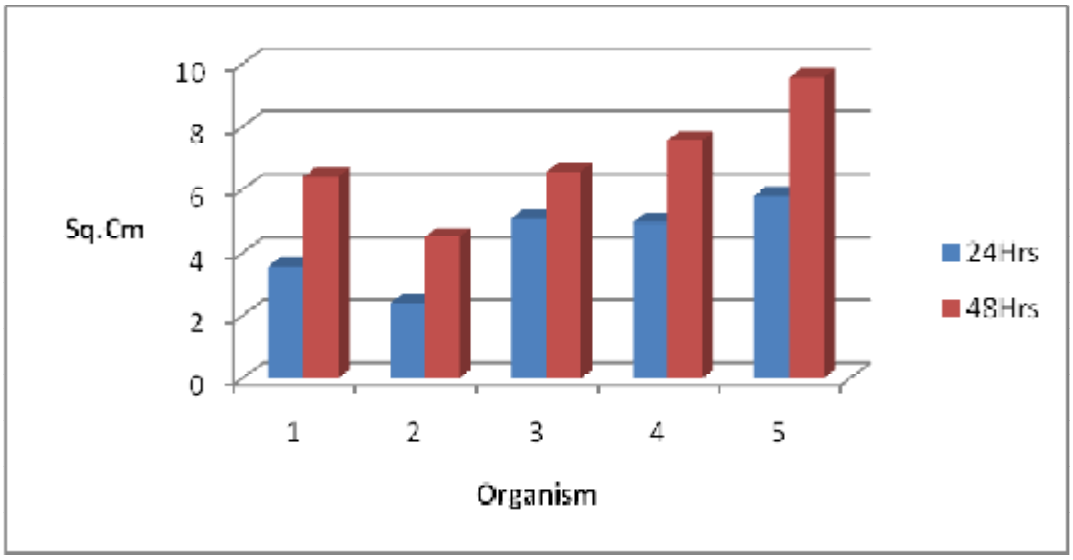
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	123.137	4	30.784	31.684	0.001
With in Groups	38.864	40	0.972		
Total	162.001	44			

TABLE16 - POST HOC TEST (48 HOURS)

Organism(I)	Organism(J)	Mean Difference (I-J)	Std. Error	Sig.
Organism 1	Organism 2	1.89889	0.46466	0
	Organism 3	-0.15778	0.46466	0
	Organism 4	-1.15778	0.46466	0.291
	Organism 5	-3.17	0.46466	0.155

* The mean difference is significant at the .05 level.

Tables 14,15 and 16 shows the difference in zone of clearance produced by 5 organisms in 48 hours. In the table 5 the F value 31.684 for the mean difference in the zone of clearance among the 5 organisms is significant.($p < .001$). Hence there is a significant difference in the zone of clearance among the 5 organisms. Further the post hoc test (table 16) reveals that the organism 2 *(Propionibacterium acnes)* has less zone of clearance compared to other organisms. The zone of clearance of organism 1,3,4, and 5 are almost similar.

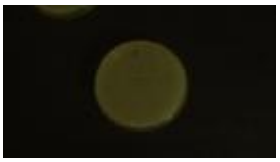


A Comparison Of Zone Of Clearance Produced By Five Organisms

RESULTS -24 Hours

Group I –Enterococcus faecalis

1a –Stock



1b -1/25th Dilution

1c -1/625th Dilution

Group II –*Propionibacterium acnes*

2a – Stock

2b -1/25th Dilution

2c -1/625th

Group III –*Actinomycosis viscosus*

3a –Stock

3b -1/25th

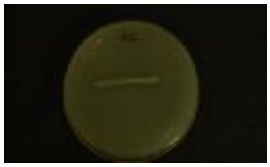
3c -1/625th

Group IV –*Pseudomonas aeruginosa*

4a –Stock

4b -1/25th DilutionDilution

4c -1/625th Dilution



Group V –*Candida albicans*

5a –Stock

5b -1/25th Dilution

5c -1/625th Dilution

RESULTS -48 Hours

Group I –Enterococcus faecalis

1a –Stock

1b -1/25th Dilution

1c -1/625th Dilution

Group II –Propionibacterium acnes

2a –Stock

2b -1/25th Dilution

2c -1/625th Dilution

Group III –Actinomycosis viscosus

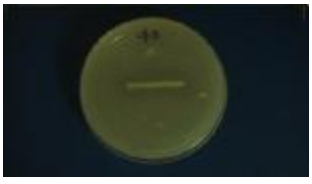
3a –Stock

3b -1/25th Dilution

3c -1/625th Dilution

Group IV –*Pseudomonas aeruginosa*

4a –Stock



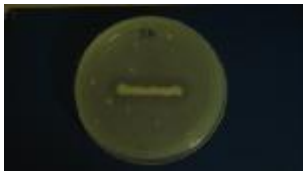
4b -1/25th Dilution

4c -1/625th Dilution

Group V –Candida albicans

5 a –Stock

5b -1/25th Dilution



5c -1/625th Dilution

Discussion

The objective of endodontic filling is to provide an inert seal after cleaning and shaping of the root canal space [19]. Gutta percha, a relatively inert thermoplastic root filling material, has been used successfully with a variety of non adhesive root canal sealers for obturation of root canals [17] .However, it lacks the adhesive properties that are necessary to seal root canal spaces. Resilon, a thermoplastic resin composite having difunctional methacryloxy groups, is capable of coupling to self etching dentin adhesives and resin cement type sealers [41,45] and reportedly produces a better seal than gutta percha against bacterial leakage [41]. Resilon is a highly filled, thermoplastic resin composite designed as a root filling material comprising of 25 % to 40% polycaprolactone and 3% to 10 % dimethacrylates [32].

Polycaprolactone was one of the earliest polymers synthesized by the Carothers group in the early 1930s [26] .It is a hydrophobic, semicrystalline polymer with good rheological and viscoelastic properties which renders it easy to manufacture and manipulate into a large range of scaffolds, including root canal points [26] .

The main core for Resilon is polycaprolactone which is a hydrophobic, biocompatible and biodegradable polyester commonly used for bioresorbable sutures and scaffolds and for micro and nanoparticulate drug delivery systems [10,12,24,46]. Polycaprolactone, is a white semi crystalline, thermoplastic, linear aliphatic polyester which is synthesized by the ring opening polymerization of caprolactone [7,35]. Polycaprolactone is a biodegradable polymer obtained from petroleum derived products. It is highly flexible and biodegradable and is finely soluble in acetone, ethylacetate,

dichloromethane, chloroform, and toluene. It is insoluble in methanol, isopropyl alcohol and in n-hexane.[9]

Incorporation of polycaprolactone as the major polymeric component in Resilon has raised the concerns regarding its potential biodegradability by salivary and bacterial enzymes that are capable of hydrolyzing ester bonds.[21]

Although the Resilon 'monoblock' system has been associated with less apical periodontitis because of its reported resistance to coronal microleakage,[41] historical appraisal of the effect of overextension of Resilon into the periapex has not been reported. The original intention of incorporating polycaprolactone in Resilon was to create a thermoplastic root filling material that is capable of resorption on inadvertent extrusion through the periapex.[21]. Thus, it is possible that biodegradation of Resilon may occur in the event of apical or coronal leakage, further compromising the seal achieved after endodontic therapy.[21]

Previous studies have shown that Resilon is susceptible to abiotic degradation via alkaline hydrolysis [14] and degradation by bacterial derived enzymes[15,22] . In these studies, gutta percha was comparatively inert against enzymatic and biotic degradation. Break of apical and coronal seals in root canal fillings may result in the penetration of these gaps by microorganisms and saliva. Hydrolases such as lipases released by bacteria, yeast and fungi can cleave the ester bonds of polycaprolactone [20, 25,13] with breakdown products utilized by the microbes as a carbon and energy source.[23]

The current study evaluated the susceptibility of Resilon to enzymatic degradation by endodontically relevant bacteria. The ester bond of polycaprolactone can be cleaved by enzymes present in the saliva or extracellular enzymes from microbes such as *Pseudomonas aeruginosa*, *Enterococcus faecalis,* and various *Actinomyces* strains [40,38,42].

In recent years, *Enterococcus faecalis* in particular has attracted considerable interest as a potential pathogen, in that it is a common isolate in root canals of failing treatments [29,44] and obviously one of the hardest organisms to eliminate once it has established itself in root canals [6]. *Propionibacterium* species have been cultured from deep layers of infected root canal dentin and has the ability to penetrate into dentinal tubules [3]. These species produce proinflammatory enzymes lipase with the capacity to support direct contact to host [34]. *Pseudomonas aeruginosa* produce true lipases and have been isolated from teeth where endodontic therapy has been compromised and is grossly inadequate [40]. *Actinomyces* species have been recovered from primary root canal infections [5,44] and secondary root canal infection non responsive to conventional treatment [6] and have been observed to degrade polycaprolactone [36] . *Candida albicans* was found in two of six specimens with therapy resistant root canal infections [31] and produces phospholipases that are concentrated at the tips of fungal hyphae and localized in the vicinity of host cellular compartment where active invasion is occurring [48, 37] .

Hence these five organisms i.e. *Enterococcus faecalis, Propionibacterium acnes, Actinomyces viscosus, Pseudomonas aerginosa* and *Candida albicans* were used in this study to assess their ability for biodegradation of Resilon.

In the present study Resilon was dispersed as emulsion which is not the way in which this material is employed as an obturating material. The feasibility of dissolving the water insoluble Resilon as aqueous emulsions and enzymatic clearing of these turbid emulsions[22] provide a potential means for incorporating emulsified Resilon in agar plates for agar well diffusion assay of enzymatic activities. Such a technique is comparable to studies that incorporate starch, casein or chitin in agar gels for enzyme activity assessment [47, 50]. These clear zones are visibly discernible without the adjunctive use of stains, fluorescent dyes or chemical binding agents as the depolymerized Resilon components are sufficiently translucent for the clearing zones to be delineated from the adjacent turbid agar medium [22].

In our study diffusion of the enzyme through the agar and the subsequent enzymatic hydrolysis of the emulsified Resilon were manifested by the loss of turbidity and the formation of clear zones around the streaks. The zone of clearance produced by all the five microorganisms was statistically significant than the negative control ($p<0.0001$) which showed no clearance around the streaks. Formation of these clear zones represents the combined results of fluid diffusion through the agar as well as the enzymatic degradation of the polymer that was dispersed within the agar.

For each substrate concentration, serial dilutions of the emulsified substrates resulted in increasingly larger clear zones that became significantly different when the Resilon emulsions were diluted to 1/25 and 1/625 times the original concentrations .The zones of clearance in all the groups were significantly more at 48 hours than 24 hours showing increased diffusion of the enzyme through the agar with time. *Propionibacterium acnes* showed the least zone of clearance in comparison to the other

four micro organisms at both 24 hours and 48 hours and the values were statistically significant at $p < 0.05$. *Candida albicans* showed highest zone of clearance at both 24 hours and 48 hours, though this difference was not statistically significant.

Though studies have been done to study Resilon degradation using enzymes derived from micro organisms, no study has reported ability of endodontic micro organisms to degrade Resilon . The findings of this study are similar to these of **Hiraishi** [22] et al who found that both polycaprolactone and Resilon were susceptible to degradation by lipase PS and cholesterol esterase enzyme using a turbidimetric analysis and optical density measurements.

The findings are also similar to another study by **Hiraishi** et al**,** where they used agar well diffusion method to test biodegradation of Resilon and polycaprolactone by cholesterol esterase, an enzyme derived from *Pseudomonas* species [21]. Positive enzyme activity was seen as zones of clearance around the wells which increased in area with increasing dilutions of the substrates in agar. Similar to their study, the sensitivity of the results was improved in our study by diluting the concentration of emulsified Resilon within the agar.

Neither gutta percha nor Resilon seal the root canal. Both require a sealer. The unique ability of Resilon to bind to the sealer and then the sealer to bind to cleaned surfaces of the dentin wall decreases available space for bacterial penetration. There is a lack of clinical data to support the laboratory findings of bacterial biodegradation of Resilon. Further studies should be carried out to test the biodegradability of Resilon bound to its sealer in an environment that mimics the root canal.

Summary &Conclusion

The present study evaluated the susceptability of Resilon to degradation by endodontically relevant bacteria like *Enterococcus faecalis, Propionibacterium acnes, Actinomyces viscosus, Pseudomonas aeruginosa* and *Candida albicans.* Resilon pellets that were dispersed into nanoparticles and were serially diluted to obtain $1/25^{th}$, $1/125^{th}$, and $1/625^{th}$ dilutions. The stock and the serial dilutions were incorporated into Brain Heart Infusion agar and then poured in sterile petridishes. The study included five groups, each group denoting an organism. Group 1- *Enterococcus faecalis,* Group 2 -*Propionibacterium acnes,* Group 3- *Actinomyces viscosus*, Group 4- *Pseudomonas aeruginosa*, Group 5- *Candida albicans* and one control group that is Group 6- Negative control which were further subdivided into subgroups A, B and C.

A-BHI agar containing stock emulsions of Resilon.

B-BHI agar containing1/25 th dilution of Resilon emulsion.

C-BHI agar containing 1/625 th dilution of the Resilon emulsion.

All these five organisms were inoculated individually as a single streak on the Resilon incorporated agar and incubated at 37^{0} C. The zones of clearance around the streaks were measured at 24 hours and 48 hours and analyzed statistically by one way ANOVA followed by post hoc and paired t test.

All microorganisms showed significant zone of clearance and comparatively higher zone of clearance in higher dilutions. The zone of clearance at 48 hours was comparatively larger than at 24 hours. *Propionibacterium acnes* showed significantly lesser zone of inhibition than other microorganisms. Further studies should be done to test the biodegradability of Resilon bound to its sealer in an environment that mimics the root canals.

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